REMARKS

Applicants respond to each rejection in paper 13 as follows.

I. 35 U.S.C. §103

There is one prior art rejection and it applies to original claims 44-45, 47-49, 59-64, 66-69, 72-76, 84-89, 93, and 95-100. The remaining original claims were not subject to any prior art rejection. In part I.A., Applicants comment first on the claims that, after amendment, are independent claims that are identical to original claims that were not subject to any prior art rejection, as well as claims dependent from such independent claims. In part I.B., Applicants respond to the prior art rejection.

A. Amended claims not subject to prior art rejection – claims 46, 50-54, 65, 70-71, 77-83, 90-92, and 94.[1]

There is no prior art rejection of any of the above-listed claims. To simplify prosecution, applicants present new independent claims 101-109 corresponding to original dependent claims 46, 50, 51, 65, 70, 71, 77, 90, 91, 92, and 94. Those dependent claims have been re-written to include the limitations of their respective base claims as shown on the following chart. The chart also shows amendments to other claims in order to change dependencies in view of the cancellation of claims, to make consistent editorial changes as detailed in part II, below, and to clarify the claimed invention.

[1] Original claims 55-58 were not subject to a prior art rejection. Since those claims have been amended to change their dependency, these remarks treat the amended claims in part I.B., below

Orig. dependent claim	New claim	Orig. dependent claim	New claim
46 (canceled) + original claim 44	101	78/107	78 amended
50 (canceled) + original claims 44, 45, and 49	102	79/107	79 amended
51 (canceled) + original claim 44	103	80/107	80 amended
52/103	52 amended	81/107	81 amended
53/103	53 amended	82/107	82 amended
54/103	54 amended	83/107	83 amended
65 (canceled) + original claims 44, 59 and 60	104	90 (original) + original claims 44 and 59	108
70 (canceled) + original claims 44, 59, 60 and 66	105	91 (original) + original claims 44 and 59	108
71 (canceled) + original claims 44, 59, 60, 66 and 67	106	92 (original) + original claims 44 and 59	108
77 (canceled) + original claims 44 59, and 75	107	94 (original) + original claims 44 and 59	109

Claims 52-54, 78-83, and 101-109 are allowable over the art.

B. Prior art rejections -- claims 44, 45, 47-49, 55-58, 60-64, 66-69, 72-76, 84-89, 93, 95-100

Each of original claims 44, 45, 47-49, 60-64, 66-69, 72-76, 84-89, 93, 95-100 was rejected as obvious from the combination of Johns et al. *Inf. Immun.* 17:9-15 (1977) and Gram et al. U.S. 5,858,728. Applicants respond to those rejections below. The discussion also applies to claims 110-111, which are new claims, and to claims 55-58, whose dependency has been changed in this amendment (see note 1, above).

1. Claims 44 (and claims that depend on it); claims 95 and 96

Applicants have discovered that LPS antigens of rough complete core mutant strains are particularly useful for active vaccination against endotoxemia. Applicants have further discovered that the vaccine should include specific detailed features. For example, claim 44 and claims that depend from it,[2] specify that the vaccine includes a cocktail of at least two such rough complete core LPS antigens from specified bacterial classifications. Independent claims 95 and 96 feature vaccines with antigen of rough complete core strain of *E. coli* K12 or active vaccination with an Rb chemotype antigen.

[2] Claims 47-49, 55-58, 60-64, 66-69, 72-76, 84-89, 93, 99-100, and 110-111.

These claim features are discussed briefly before reviewing the prior art rejection.

a. Active vaccination

Active vaccination involves only the immune system of the vaccine recipient, with no selection or ex-vivo amplification of the recipient's immune response. For example, in active vaccination, there is no opportunity to identify and amplify a specific desired immune response, e.g., through selection and amplification of one hybridoma that produces a desired antibody. The claims language is clear that the vaccine protects the individual animal to whom the vaccine is administered, so the claims are limited to active vaccination.

b. Rough complete core LPS; cocktails; **E.coli** *K12; Rb chemotype*

The specification explains what rough complete core LPS mutants are. See in particular page 3, line 15 through page 4, line 30 and Figs. 1-3 of WO98/09988.[3] Rough LPS mutants are those lacking the O-side chain polysaccharide. The core is illustrated in Fig. 2. Clearly, Re mutants, e.g., Re mutants of *S. minnesota*, are not rough complete core mutants.

Regarding the more detailed invention of claim 44 (and its dependent claims), vaccination features a cocktail that includes LPS antigens from rough complete core mutant bacteria from the following classifications: *E. coli*; *Pseudomonas; Klebsiella; Salmonella;* and *Bacteroides*. Claims 95 and 96 specify vaccination with rough complete core mutants of a specific strain, *E. coli* K12, or with Rb chemotype mutants.

Having described the claim elements, we now review the cited art.

c. Johns et al. does NOT teach active vaccination using LPS of multiple rough complete core mutants

Applicants specifically take issue with the sentence that is critical to this rejection (page 6, lines 3-5 of paper 13), characterizing Johns et al. as,

[3] PCT WO98/09988 is the PCT publication in the international phase of this U.S. national phase patent application.

> "teach[ing] administration of rough mutants of *S. minnesota* Ra, Rb, ... to animals, producing antibodies which bind to the core LPS...."

In fact, Johns et al. does not report any administration Ra or Rb chemotype mutants to animals, let alone a cocktail of such mutants or LPS from such mutants. Since it does not show administration of a composition comprising LPS from Ra or Rb chemotype mutants to animals, it of course does not show that "such administration is protective" (paper 13, page 6, line 5). The citation in paper 13 for the above-quoted characterization of Johns is page 15, 3'rd paragraph, first column. That discussion concerns experiments administering immunogens of mutant bacilli (specifically Re mutants), or simply isolated lipid A (which lacks the polysaccharide component of LPS altogether). Those immunogens have nothing to do with the immunogens from rough complete core mutants specified in the claims.

Perhaps the examiner has focused on an experiment reported in Johns et al., which was designed to find out whether the Johns et al. immunogens (smooth bacilli, Re chemotype or lipid A) would produce antibodies that cross-react generally with gram negative LPS antigens, such as those of Ra, Rb, and Rc LPS. Thus, at page 12, Johns et al. says,

> Antisera obtained after **immunization with smooth bacilli, Re mutants** or lipid A were screened for antibody titers to LPS from a number of heterologous bacteria....As may be seen significant rises in antibody titer were observed to [various listed bacterial strains, some of which are rough, complete core strains].

Johns et al. wanted to know whether antisera obtained by vaccination with smooth bacilli, with Re mutants, or with lipid A would cross-react with other bacteria. The article does not disclose or suggest immunization with antigens of any bacterial strains other than those of smooth bacilli, Re chemotype or lipid A.

So Johns et al. says nothing about the claimed use of LPS immunogens from rough complete core mutants as an active vaccine.

Interestingly, the teaching in Johns et al. to vaccinate with immunogens from Re mutants (rather than from rough complete core mutants according to the claims) is a theme repeated in other papers from the same laboratory. An earlier paper by a co-author of Johns et al. (McCabe, *J. Immunology*, 108:601-6110 (1971)),[4] reports cross-reactivity studies comparing cross-

[4] McCabe is enclosed herewith as reference GS.

reactivity of serum produced by vaccination with Ra LPS to cross-reactivity of serum produced by vaccination with incomplete core LPS from mutants such as Re. The authors conclude that only by stripping off the core sugars from complete core mutants such as Ra (see Fig. 2 of the Applicants' specification) can they obtain an immunogen that presents common features shared more generally by gram-negative bacteria. At p. 609, McCabe concludes,

> Presently, only the Rd and Re mutants of various species of Gram-negative bacilli are felt to have identical compositions. Thus, only Rd_1, Rd_2 and Re mutants actually contain antigens completely shared by the challenge strains. ....these minor differences in the composition of the core region may explain **the failure of Ra, Rb and Rc mutants to induce protection**, ...

The teaching of McCabe, followed by Johns et al., is that the smaller the core antigen (i.e., the less complete it is), the more it has in common with other Gram negative strains, and, therefore, the better it will be at eliciting protection that is cross-reactive.

In sum, Johns et al. does not teach active vaccination with a composition comprising rough, complete core LPS antigen, let alone a cocktail of several of such antigens according to claim 44. Nor does Johns et al. teach vaccination with *E. coli* K12 rough complete core antigen according to claim 95 or Rb mutants according to claim 96.

d. The art provides no expectation of success for the proposed combination of Johns et al. with Gram et al.'s disclosure related to immunization with monoclonal antibodies.

The rejection combines Johns et al. with another reference, Gram et al., disclosing the production of carefully selected monoclonal antibodies for use in passive methods of protection. In Gram et al., the patient does not receive a vaccination; the patient receives a composition prepared in the laboratory. The combination is explained (paper 13, page 6, lines 10-12),

> ...it would have been obvious...to modify the teachings of Johns et al. and Gram et al. to directly administer the rough, complete-core LPS mutants to protect against endotoxemia in recipients.

First, as we have seen, the office action is not accurate in describing the teaching of Johns et al. because Johns et al. does not teach administering rough complete core mutants at al.

Moving on from that deficiency in the rejection, what does Gram et al. teach about rough complete core mutants? Gram et al. elects an entire different approach from the claimed invention. Mice are immunized with a cocktail of complete core mutants, but NOT with the expectation that the immunized animal will produce a protective immune response. Instead, the immunized animals are used as a source of spleen cells that are harvested, immortalized and then subjected to careful selection to find a few or even one cell that has the desired properties. Once found, the desired single cell is amplified to produce a monoclonal antibody administered to a patient.

In Example 4 (col. 18 line 53 through col. 19, line 55) of Gram et al., the authors report immunization of five mice. One out of those five mice was selected based on serum response. Then that one mouse was given a booster immunization. Finally, 260 hybridomas were produced from the carefully selected animal. Only twenty of these hybridomas (fewer than eight percent) produced a strong response. The 20 selected hybridomas were screened further to produce a single clone from which a single monoclonal antibody was obtained.

As noted, the claims specify active vaccination intended to protect the animal receiving the immunogen. In contrast, the careful screening process used in Gram et al. was necessary precisely because of doubt about the capacity of the serum of an immunized animal to afford protection. Protection of the mice was not expected and not tested—i.e., none of the mice was challenged to determine protection. Certainly protection of the four mice that were not examined further was not expected. Protection was not even expected for the one mouse that was selected. The fact that a single spleen cell produces a "useful" antibody does not establish protection of the animal from which the cell was taken was protected, and, as noted below, the monoclonal antibody procedure is used in part because protection is not expected without it.

An obviousness rejection must be based both on a finding of prior art motivation or suggestion to try the proposed combination and on the expectation that the combination would succeed. For example, in *Amgen, Inc. v. Chugai Pharmaceutical Co. Ltd.*,[5] the U.S. Court of Appeals for the Federal Circuit concluded that, while a particular approach to cloning the human erythropoietin gene "may have been obvious to try, the realization of that idea would not have

[5] 927 F.2d 1200, 18 USPQ2d 1016 (Fed. Cir. 199), *cert. denied*,501 U.S. 856 (1991).

been obvious." Similarly, in its decision in *In re Vaeck*,[6] the Federal Circuit reversed the U. S. PTO's decision that prior art showing expression of an antibiotic resistance gene in cyanobacteria made it obvious that other unrelated genes, such as genes encoding insecticidal proteins, could be expressed in cyanobacteria. The Federal Circuit concluded that the prior art did not disclose a reasonable expectation of success for the expression for insecticidal proteins in cyanobacteria and for that reason it reversed the PTO's rejection of Vaeck's patent application.

As in *Amgen* and *Vaeck*, the art in this case lacks any support for the expectation that merely finding an antibody-producing cell (among many) in an immunized animal predicts that the animal from whom the cell was taken is protected. In fact, Gram et al. indicate that the motivation to use monoclonal antibodies lies in the "limited efficacy" of serotherapy (col. 2, lines 21-51),

> To overcome the limited efficacy of serotherapay, it has been proposed to use cross-reactive MAbs....A MAb expected to be useful for preventing or treating bacteremia should not only be cross-reactive but also cross-protective against the infections caused by the most common toxic bacteria....
>
> ***
>
> However, it has been reported in several scientific articles....that the large majority of antibodies raised against the conventional immunogens cited above cross-react poorly and, unfortunately fail to be protective against infection.

So Gram et al. itself teaches away from the expectation of success for active vaccination that is required for an obviousness rejection.

e. Conclusion

First, the basis for the rejection--that Johns et al. vaccinated with a composition comprising rough complete core mutants—is inaccurate. Second, Gram et al. does not deal with active vaccination at all. Third, the Examiner has not cited any basis in the art to expect the proposed combination to succeed.

Specifically as to claim 44 and its dependent claims, those claims further recite active vaccination with a cocktail of the rough complete core LPS mutants from specific strains. Claim

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[6] *In re Vaeck*, 947 F. 2d 488, 20USPQ2d 1438 (Fed. Cir. 1991).

44 and its dependent claims are allowable over the art based on the features they cite in addition to those of claim 44.

Claims 95 and 96 are allowable over the art for the additional reasons that they recite, respectively, a vaccine comprising a specific complete core rough strain, *E. coli* K12 and active vaccination with Rb chemotype antigen.

It is respectfully pointed out that the obviousness rejection of claims 44, 95 and 96 (and claims dependent from them) must be withdrawn in view of the facts and law cited above.

2. Claims 97 and 98

Claims 97 and 98 feature methods of quantitating LPS incorporated into liposomes. It is not clear from the office action how the cited art applies to these claims.

II. 35 U.S.C. §112 ¶2 – paragraphs 7-8 of paper 13

Claims 44 and 45 are specifically mentioned in this rejection.

Claim 44 has been amended to clarify that the method uses the rough complete core antigen of multiple gram-negative bacterial strains chosen independently from the list of bacterial classifications originally presented in claim 45. In making this amendment to claim 44, a semicolon has been inserted between *Salmonella* and *Bacteroides*. Basis for the use of antigens from more than one bacterial strain can clearly be found in claim 45 (now canceled because it is combined with claim 44) and in the specification as filed, for example at page 7 lines 32 through page 8, line 28 which discusses a cocktail that include complete core rough LPS antigens from multiple different gram negative bacterium.

This amendment also makes clear that the composition includes LPS from the specified bacterial strains, avoiding misuse of the term "species" from earlier claims and avoiding an unintended claim interpretation—ie., that the claims specify the use of immunogen from a single bacterial cell. Corresponding amendments have been made to a number of other claims. Basis for these amendments is found, among other places, in claim 74, which correctly refers to bacterial strains.

The phrase "further comprises" now clearly refers to a component in the claimed composition in addition to components specified in the parent claims.

Other claims have been amended for consistency and claims 110 and 111 have been added to more completely claim the invention.

These amendments overcome the rejections in paragraphs 7-8 of the office action.

III. 35 U.S.C. §112 ¶1 – paragraph 9 of paper 13

The enablement rejection covers all pending claims except claims 97-98 and it is based on the conclusion that, while the specification enables immunizing, it does not enable *in vivo* treatment. The rationale for this distinction is the absence of examples for *in vivo* treatment (page 4, line 3 through page 5, line 7 of paper 13),

> "...the specification, while being **enabling for** producing antibodies by **immunizing** with compositions comprising rough complete-core LPS, does **not** reasonably provide enablement **for treatment** of animals by reducing the adverse effects of endotoxin.....The ... invention [is] methods of reducing the adverse effects of endotoxin in warm blooded animals, i.e., *in vivo*... the specification is **silent concerning examples of the treatment of animals** to reduce the adverse effects of endotoxin. The specification teaches antibody production and in vitro binding assays. The specification [sic contains?] no examples concerning the dosage, timing of administration, etc. which are the necessary parameters for determining the proper steps to fulfill the methods claimed.

There can be no question that the specification teaches the art how to immunize animals; thus the rejection is based on the absence of examples establishing *in vivo* therapy.

A. The mouse lethality model

Applicants point out that the specification includes a well-established mouse lethality model, described at page 34, lines 10-37 of the specification.[7] Adequate information about dosage and timing of administration for this model is provided in specification. For example, the specification teaches the animals are vaccinated on day 0 and again on days 7 and 14 (34:13-19 of WO '988). Formulation of various vaccine cocktails are detailed in Examples 1 and 2 at

[7] All references to the specification are to the international publication for this U.S. national phase application, (WO98/09988).

pages 35-36 of WO '988. Methods for verifying the presence of the immunogen in the vaccine are provided at page 24:17 through 25:7. Further details about vaccination will be apparent from the rabbit examples at 25:8-26:19.

In the mouse lethality model, mice are subjected to a lethal challenge with LPS – i.e., a does of LPS that is lethal (34:23-26 of WO '988). Such a dosage is readily determined by experiments in which increasing doses are administered and lethality is determined at 24 hours (Id).

In fact, the mouse lethality experiments laid out in the specification do establish that the invention is protective. Accompanying this response as APPENDIX A is a copy of an article (Bennett-Guerrero et al., INFECTION AND IMMUNITY, vol. 68, pp.6202-2308 (2000)) coauthored by the inventors. The article reports that mice immunized with complete core LPS were protected against a lethal challenge with gram-negative bacterial endotoxin. The mouse model is detailed at page 6204 of the article, under **"Mouse lethality:galactosamine (D-GalN) model"** and the results of that experiment are presented at page 6205 and in FIG 5. The discussion bridging from p. 6206-7 makes clear that the antibodies elicited in the vaccinated mice conferred protection on those mice (Fig. 5) following challenge with LPS from E. coli O18, a serotype accounting for 27% of fatal cases of *E. coli* bacteremia as reported by Gransden et al. (ref. 23). In short, the mouse lethality model is well accepted and it demonstrates that the detailed guidance provided in the specification is adequate to practice the claimed invention.

B. Rabbit serology examples

Applicants also point out that the specification describes in detail experiments showing that administration of complete core rough LPS antigens to rabbits provides an immune response that is cross-reactive with strains and species that were not part of the immunization. Sera from immunized rabbits reacted with numerous clinically relevant gram-negative bacteria. Pages 25-26 of the specification describe an experiment in which rabbits are immunized and tested for the production of antibodies that bind to LPS. [Such serology experiments are reported in FIGS. 3 and 4 of the enclosed Bennett-Guerrero et al., article, APPENDIX A.] Thus, experiments detailed in the specification as filed establish that vaccination of rabbits produces antibodies not

previously present, and those antibodies react with a broad panel of gram-negative bacterial genera.

In sum, reconsideration of this rejection is respectfully requested in light the amendments to the claims and in light of Bennett-Guerrero et al., APPENDIX A.

IV. Request to consider prior art.

The office action indicates that a number of prior art references have not been considered because they were not supplied with the form 1449 listing those references. Several different issues are raised.

A. References not considered because no copies were available to the examiner.

In most cases, the reason indicated for not considering the references was that the examiner did not receive a copy of the reference. Applicants submitted three separate Information Disclosure Statements, and postcard receipts were submitted with each one. Enclosed with this response are the stamped receipts. These receipts indicate that most of the references not considered were received at the U.S. PTO mailroom.

B. Incomplete reference

In one case, a reference was not considered because it was incomplete. A complete copy of that reference is enclosed.

C. References in German with English abstracts

In two cases, references were not considered because no translation was submitted. In each case, the reference submitted was an article with an English abstract and German text.

D. The enclosed Form 1449

Applicants enclose a new form 1449 listing each reference to be considered. Copies of each listed reference accompany this response. The listing for the documents that were combined English and German has been revised to clarify that the examiner has considered only the English abstract.

Accordingly, Applicants respectfully request that these references be considered, and that the enclosed form 1449 be initialed to indicate such consideration.

CONCLUSION

Applicant asks that all claims be allowed. Please apply any other charges or credits to Deposit Account No. 06-1050.

Attached is a marked-up version of the changes being made by the current amendment.

Respectfully submitted,

Date: 12/12/01

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claims 45-46, 50-51, 60, 65, 70-71, and 77 are canceled.

Claims 44, 47-49, 52-55, 57-59, 61, 64, 66-69, 72-73, 75, 78-85 and 88-93 are amended.

Claims 101-111 are new.

44. (Amended) A method of reducing adverse effects of endotoxin in a warm-blooded animal, which comprises administering to the [warm-bloooded] warm-blooded animal an effective amount of a composition comprising rough, complete-core lipopolysaccharide (LPS) antigens of [a] at least two G[g]ram negative [bacterium] bacterial strains, each of said strains having a classification independently selected from the following classifications: *E. coli*; *Pseudomonas; Klebsiella; Salmonella*; and *Bacteroides*.

47. (Amended) The method of claim 44 in which the composition comprises killed whole cells of said bacterial strains.

48. (Amended) The method of claim 47 in which the composition comprises killed whole *E. coli* K12[,] cells containing rough complete-core LPS antigen.

49. (Amended) The method of claim [45] 44 in which the composition comprises a cocktail of killed Ra chemotype [whole-cell mutants] bacterial cells classified in [of] at least three of the following [species] classifications of G[g]ram-negative bacteria: *E. coli* K12, *E. coli* R1, *Bacteroides fragilis*, *Pseudomonas aeruginosa*.

52. (Amended) The method of claim [51] 103 in which the Ra LPS conjugate is [conjugated] *E. coli* K12 Ra LPS conjugate.

53. (Amended) The method of claim [51] 103 in which the composition comprises a cocktail of Ra LPS antigens from multiple [species] strains of gram-negative bacteria, said antigens being conjugated to a protein.

54. (Amended) The method of claim 53 in which the composition comprises conjugates of Ra LPSs from at least three strains [of the following species] of G[g]ram-negative bacteria, each of said three strains being classified in a different one of the following classifications: *E. coli* K12, *E. coli* R1, *Bacteroides* fragilis, *Pseudomonas aeruginosa.*

55. (Amended) The method of claim [53] 44 in which the composition comprises Ra LPS incorporated in a liposome.

56. The method of claim 55 in which the composition comprises E. coli K12 Ra LPS in a liposome.

57. (Amended) The method of claim 55 in which the composition comprises a cocktail of Ra LPSs from multiple species of G[g]ram-negative bacteria incorporated in liposomes.

58. (Amended) The method of claim 57 in which the cocktail comprises Ra LPSs from at least three strains [of the following species] of G[g]ram-negative bacteria, each of said strains being classified in a different one of the following classifications: *E. coli* K12, *E. coli* R1, *Bacteroides fragilis*, and *Pseudomonas aeruginosa.*

59. (Amended) The method of claim 44 in which [the composition comprises rough, complete-core lipopolysaccharide (LPS) antigen of] one of said bacterial strains is classified as *E. coli* K12.

61. (Amended) The method of claim [60] 59 in which the animal is a mammal.

64. (Amended) The method of claim [60] 59 in which the [second] other of said bacterial strains is classified as [bacterium is an] *E. coli* or [a] *Salmonella* [bacterium].

66. (Amended) The method of claim [60] 59 in which the composition comprises complete-core, rough, LPS antigen from a third Gram-negative [bacterium] bacterial strain different from the first and from the second Gram-negative [bacterium] bacterial strains.

67. (Amended) The method of claim 66 in which the composition comprises complete-core, rough, LPS antigen from a fourth Gram-negative [bacterium] bacterial strain different from each of the first, the second, and the third Gram-negative [bacteria] bacterial strains.

68. (Amended) The method of claim 59 in which the other of said [second] Gram-negative [bacterium] bacterial strains is *E. coli* R1.

69. (Amended) The method of claim 59 in which the other of said [second] Gram-negative [bacterium] bacterial strains is a *Salmonella* [bacterium].

72. (Amended) The method of claim 64 or claim 69 in which the *Salmonella* [bacterium] strain is *Salmonella minnesota* .

73. (Amended) The method of claim 67 in which complete core antigen from each of the four [bacteria] bacterial strains is present in generally equal amounts by weight.

75. (Amended) The method of claim 59 in which the antigens cause[s] the patient to produce an antibody that binds to an epitope in the core region of the LPS of at least one Gram-negative bacterial strain whose LPS is not part of the composition.

78. (Amended) The method of claim [77] 107 in which the ratio (weight:weight) of lipid in the liposome to the LPS antigens is between 1:1 and 5000:1.

79. (Amended) The method of claim [77] 107 in which the ratio (weight:weight) is between 10:1 and 1000:1.

80. (Amended) The method of claim [77] 107 in which the liposome comprises a component selected from the group consisting of: phospholipid, cholesterol, positively charged compounds, negatively charged compounds, and amphipathic compounds.

81. (Amended) The method of claim [77] 107 in which the liposome is a multilamellar type liposome (MLV).

82. (Amended) The method of claim [77] 107 in which LPS in the acid salt form is incorporated into the liposome.

83. (Amended) The method of claim [77] 107 in which the liposome is a small or large unilamellar liposome (SUVs and LUVs).

84. (Amended) The method of claim [59] 44 in which the composition is administered intramuscularly, intravenously, subcutaneously, intraperitonealy, via the respiratory tract, or via the gastrointestinal tract.

85. (Amended) The method of claim [59] 44 in which the dose of antigen is over 0.01 ng per kilogram of patient body weight.

88. (Amended) The method of claim [59] 44 in which the composition is administered in multiple doses, the first of which is administered at least 2 days prior to potential endotoxin exposure.

89. (Amended) The method of claim 59 in which the antigen is present in a killed [bacterium] bacterial cells.

90. (Amended) The method of claim [59] 44 in which the antigen is separated from [the bacterium] bacterial cells.

91. (Amended) The method of claim [59] 44 in which the antigen is chemically detoxified.

92. (Amended) The method of claim [59] 44 or claim 90 in which the bacterium is genetically engineered.

93. (Amended) The method of claim [59] 44 in which the composition further comprises an adjuvant.

--101. A method of reducing adverse effects of endotoxin in a warm-blooded animal, which comprises administering to the warm-blooded animal an effective amount of a composition comprising rough, complete-core lipopolysaccharide (LPS) antigens of *E. coli*, of *Pseudomonas;* and of *Bacteroides.*

--102. A method of reducing adverse effects of endotoxin in a warm-blooded animal, which comprises administering to the warm-blooded animal an effective amount of a composition comprising a cocktail of killed Ra chemotype whole-cell mutants of each of the following classifications of gram-negative bacteria: *E. coli* K12, *E. coli* R1, *Bacteroides fragilis, Pseudomonas aeruginosa.*

--103. A method of reducing adverse effects of endotoxin in a warm-blooded animal, which comprises administering to the warm-blooded animal an effective amount of a composition comprising rough, complete-core lipopolysaccharide (LPS) antigen of a gram negative bacterial strain, said antigen being conjugated to protein, wherein said antigen is purified detoxified Ra LPS.

--104. A method of reducing adverse effects of endotoxin in a warm-blooded animal, which comprises administering to the warm-blooded animal an effective amount of a

composition comprising rough, complete-core lipopolysaccharide (LPS) antigen of *E. coli* K12 and rough, complete-core lipopolysaccharide (LPS) antigen of a *Bacteroides* bacterial strain.

--105. A method of reducing adverse effects of endotoxin in a warm-blooded animal, which comprises administering to the warm-blooded animal an effective amount of a composition comprising rough, complete-core lipopolysaccharide (LPS) antigen of at least one gram negative bacterium, said composition comprising:

rough, complete-core lipopolysaccharide (LPS) antigen of *E. coli* K12;

rough, complete-core lipopolysaccharide (LPS) antigen of a *Klebsiella* bacteria;

rough, complete-core lipopolysaccharide (LPS) antigen from a *Pseudomonad.*

--106 The method of claim 105 in which the composition further comprises rough, complete-core lipopolysaccharide (LPS) antigen of a *Bacteroides.*

--107. A method of reducing adverse effects of endotoxin in a warm-blooded animal, which comprises administering to the warm-blooded animal an effective amount of a composition comprising rough, complete-core lipopolysaccharide (LPS) antigen of at least one gram negative bacterium, the composition comprising rough, complete-core lipopolysaccharide (LPS) antigen of *E. coli* K12, and the antigen causing the patient to produce an antibody that binds to an epitope in the core region of the LPS of at least one Gram-negative bacterial strain whose LPS is not part of the composition, the antigen being present in a liposome.

--108. A method of reducing adverse effects of endotoxin in a warm-blooded animal, which comprises administering to the warm-blooded animal an effective amount of a composition comprising rough, complete-core lipopolysaccharide (LPS) antigen of at least one gram negative bacterium, the composition comprising rough, complete-core lipopolysaccharide (LPS) antigen of *E. coli* K12, in which the antigen is produced by one or more of the following methods: a) it is separated from the bacterium; b) it is chemically detoxified; c) it is genetically engineered.

--109. A method of reducing adverse effects of endotoxin in a warm-blooded animal, which comprises administering to the warm-blooded animal an effective amount of a composition comprising rough, complete-core lipopolysaccharide (LPS) antigen of at least one gram negative bacterium, the composition comprising rough, complete-core lipopolysaccharide (LPS) antigen of *E. coli* K12, and alum as an adjuvant.--

--110. The method of claim 64 in which the other of said bacterial strains is classified as *E. coli*.—

--111. The method of claim 44 of 64 in which the bacterial strains are classified as Ra rough mutant strains.--